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A safe lithium mimetic for bipolar disorder

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Abstract

Lithium is the most effective mood stabilizer for the treatment of bipolar disorder, but it is toxic at only twice the therapeutic dosage and has many undesirable side effects. It is likely that a small molecule could be found with lithium-like efficacy but without toxicity through target-based drug discovery; however, lithium's therapeutic target remains equivocal. Inositol monophosphatase is a possible target but no bioavailable inhibitors exist. Here we report that the antioxidant ebselen inhibits inositol monophosphatase and induces lithium-like effects on mouse behaviour, which are reversed with inositol, consistent with a mechanism involving inhibition of inositol recycling. Ebselen is part of the National Institutes of Health Clinical Collection, a chemical library of bioavailable drugs considered clinically safe but without proven use. Therefore, ebselen represents a lithium mimetic with the potential both to validate inositol monophosphatase inhibition as a treatment for bipolar disorder and to serve as a treatment itself.

Bipolar disorder affects 1-3% of the population and the most effective treatment for stabilizing mood is lithium [1]. Lithium is also the only agent that reduces suicidal thoughts and actions [2]. Unfortunately, lithium is toxic at only twice the therapeutic dosage and has many undesirable side effects including weight gain, thirst, tremor and kidney damage [3]. To develop a lithium mimetic—ideally a drug with its therapeutic action but without its disadvantages—would require an understanding of lithium's mechanism of action, which, even after six decades of use [4], remains controversial [5]. Lithium displaces magnesium ions and inhibits at least 10 cellular targets, all of which are components of intracellular signalling pathways[5]. However, targets inhibited by lithium at therapeutically relevant concentrations (0.6-1 mM) narrows the targets to two: glycogen synthase kinase 3ß[6] and inositol monophosphatase [7-9]. Both putative targets have experimental evidence for and against them based on genetics and pharmacology[6,9-12]. Additionally, several chemically distinct bipolar medications (lithium, valproic acid and carbamazapine) all have a common mechanism of action affecting the inositol cycle[13]. Inhibition of inositol monophosphatase by lithium led to Berridge's 'inositol depletion hypothesis' that suggests that Ins1P

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accumulates and inositol is depleted[7]. Given that in neurons regeneration of phosphatidylinositol 4,5-bisphosphate requires recycling of inositol from Ins1P, lithium dampens signalling in cells with overactive signalling through pathways using a G-protein-coupled receptor linked to phospholipase C[7].

IMPase remains a potential therapeutic target for bipolar disorder, but its validation requires small molecule inhibitors. However, little progress has been made in regard to inhibitors since a large effort by Merck yielded a potent (IC_{50} 300 nM) antagonist (L-690,330) but neither it nor its esterified prodrug (L-690,488) was bioavailable [14,15]. We now report that ebselen inhibits IMPase and acts as a lithium mimetic in mouse models of bipolar disorder.

Results

Repurposing reveals ebselen as an inhibitor of IMPase

To identify an inhibitor of IMPase, we expressed human IMPase in bacteria and used it in an assay to screen the NIH Clinical Collection provided through the National Institutes of Health Molecular Libraries Roadmap Initiative [16]. Compounds in this collection have a history of use in human clinical trials, are drug-like with known safety profiles and may even be appropriate for direct human use in new disease areas (www.nihclinicalcollection.com). A primary screen at 100 μM of each drug in the collection identified ebselen (Fig. 1a) as an inhibitor of IMPase, and we characterized it further with a full concentration-response curve (Fig. 1b). The potency of ebselen against IMPase (IC_{50} 1.5 μM) compared favourably to that of the known but poorly bioavailable inhibitor L-690,330[14] (IC_{50} 0.3 μM) and was greater than that of lithium (IC_{50} 0.8 mM; Fig. 1b). Importantly, the greater potency of ebselen for IMPase (Fig. 1b) compared to glycogen synthase kinase 3ß (Fig. 1c) demonstrates selectivity, making ebselen of diagnostic use in determining the therapeutic potential of IMPase inhibition.

Ebselen inhibition is irreversible and covalent

As increasing concentrations of ebselen decreased V_{max} with little effect on K_m (Fig. 1d,e) the inhibition is not competitive[17]. Inhibition of IMPase by ebselen was not fully relieved by dilution (20 μM to 0.2 μM ebselen; Fig. 1f) even after a time course for recovery extending to 25 h (Fig. 1g), thus indicating that inhibition is, for practical purposes, irreversible. (A reversible inhibitor would lose potency upon dilution due to mass action promoting dissociation[17].) As irreversible inhibition often arises from covalent binding, we looked for direct evidence of ebselen binding to IMPase. Mass spectrometry revealed that a mixture of IMPase and ebselen formed complexes heavier than pure IMPase *dimer* by the mass of one or two ebselen molecules under both denaturing and non-denaturing conditions (Fig. 1h), supporting covalent binding and 1:1 stoichiometry per *monomer*. In contrast, a mixture of IMPase and the reversible inhibitor L-690,330[14] formed heavier complexes under non-denaturing conditions, but not under denaturing conditions (Fig. 1h).

Ebselen contains selenium (Fig. 1a), which can form a selenylsulfide (–Se–S–) bond[18,19]. For bovine IMPase, alkylation of cysteine 218 with the non-selective agent N-ethylmaleimide inhibited activity[20]. In bovine IMPase, cysteine 218 is near the active site residue aspartate 220, which is required for magnesium ion coordination and catalysis[20]. The position of this cysteine is conserved in both the mouse and human isoforms based on its crystal structure[21]. To test the importance of this cysteine in mediating ebselen inhibition, we constructed a human IMPase with a cysteine to alanine mutation (C218A). The C128A mutant was indeed less sensitive to ebselen inhibition, based on the increase in IC_{50} for ebselen (Fig. 1i) and a smaller decrease in V_{max} (Fig. 1j,k). Furthermore, an analogue ebselen in which the selenium is substituted with sulphur (ebsulfur; Fig. 1a) weakly

inhibited IMPase (Fig. 1l), whereas a selenium-containing compound with similar electrophilic reactivity (dibenzyldiselenide; Fig. 1a) had no inhibitory effect (Fig. 1l). These data demonstrate that inhibition of IMPase by ebselen requires not just the presence of an electrophilic selenium atom but also an appropriate chemical scaffold. Unlike the case for most other proteins when covalently linked to ebselen[18,19,22], inactivation of IMPase was not reversed by post-incubation with the sulfhydryl reducing agents glutathione and dithiothreitol (Fig. 1m). Pre-incubation of ebselen with the reducing agents did, however, reduce inhibition (Fig. 1n) as described in detail below.

Ebselen is pharmacologically active in the brain

To determine whether ebselen can cross the blood–brain barrier and thus be pharmacologically active in mouse brain, as reported for rat[23], we exploited the irreversible inhibition property of ebselen in an *ex vivo* method based on IMPase activity in brain homogenate (Fig. 2a). As the initial experiments that identified ebselen as an inhibitor used recombinant human IMPase (Fig. 1b), we first needed to ensure that recombinant mouse IMPase was enzymatically active. Recombinant mouse IMPase was inhibited by lithium and L-690, 330 and ebselen (Fig. 2b). Having validated that ebselen inhibited the mouse form of IMPase, we demonstrated that in homogenates of mouse brain, IMPase activity was detectable and inhibited by lithium, L-690,330 and ebselen (Fig. 2c). In an *ex vivo* experiment, IMPase activity was measured in brain homogenates prepared at various times after intraperitoneal injection of ebselen (Fig. 2a)[24]. Over time, IMPase inhibition developed and then returned to control levels (Fig. 2d,e). Therefore, systemic administration of ebselen inhibits IMPase in mouse brain in whole animals.

That IMPase inhibition by ebselen was detected in the *ex vivo* experiments (Fig. 2d,e) is revealing in regard to the likely chemical form of ebselen in intact cells *in vivo*, as its selenium atom can exist in higher or lower oxidation states, and these have different reactivities[18,19]. Incubation of ebselen with reduced glutathione forms ebselen–glutathione selenenylsulphide, whereas incubation with dithiothreitol reduces ebselen to its selenol and diselenide (Fig. 1a,l)[18,19]. When we pre-incubated ebselen with these reducing agents the reduced forms of ebselen (confirmed by mass spectrometry) were weaker inhibitors of IMPase (Fig. 1n), likely because they are less reactive with cysteines[18]. Therefore, to obtain inhibition of IMPase with ebselen *in vivo* a fraction of ebselen must exist in a non-conjugated free form in the oxidation state shown in Fig. 1a, despite an intracellular environment with millimolar concentrations of reduced glutathione[25].

Ebselen alters the function of the central nervous system

To determine whether ebselen was affecting the function of the central nervous system, we investigated the effect of ebselen on the responses mediated by the serotonergic 5-HT_2 receptor[26]. In humans, lithium reduces phosphoinositide cycle-coupled 5-HT_2 receptor function[27], and this may be linked to lithium's antidepressant action. Lithium also reduces 5-HT_2 receptor function in mouse as modelled by a 5-HT_2 agonist-evoked head-twitch response[28]. This is mediated by the prefrontal cortex[29], which is believed to be the target of lithium in the treatment of bipolar disorder[30]. Ebselen decreased 5-HT_2 agonist-induced head twitches in a dose-dependent manner (Fig. 3a), and this was associated with decreased expression of *Arc* mRNA (a molecular marker of neural activity[26]) in the prefrontal cortex (Fig. 3b) and cingulate cortex (Fig. 3c). Thus, ebselen attenuates a cortically mediated 5-HT_2 receptor response that is linked to phosphoinositide turnover, as would be predicted for an inhibitor of IMPase.

Ebselen exhibits lithium-like effects on behaviour

Rodent behaviours are used to model bipolar disorder, and typically focus on either the manic or the depressive pole[31]. The 'learned helplessness' aspect of depression is often modelled with the forced swim test. In this model, ebselen has recently been shown to exhibit anti-depressant action[32]. Given these findings, we investigated the effect of ebselen in lithium-sensitive mouse models of mania[33]. In the open field test (Fig. 3d), rearing was decreased by ebselen over time and then returned to baseline (Fig. 3e), a time course that paralleled that for IMPase inhibition in the *ex vivo* assay (Fig. 2e), as well as plasma ebselen concentrations in humans after oral administration[34]. Rearing is an exploratory behaviour that correlates with impulsivity[33], which in turn correlates with suicidal thoughts and actions[35]. Mania has also been modelled by amphetamine-induced hyperactivity (Fig. 3f)[33,36]. Similarly to lithium[37], ebselen reduced amphetamine-induced hyperactivity in a manner that depended on both the dose of amphetamine and the dose of ebselen (Fig. 3g), as is the case for lithium[37]. Baseline mobility was not significantly reduced (one-tailed, paired t-tests: amphetamine 2 mg/kg and ebselen 5 mg/kg, $p=0.24$; amphetamine 4 mg/kg and ebselen 5 mg/kg, $p=0.08$).

Ebselen acts through inositol depletion

Finally, if ebselen were mimicking lithium by inhibition of IMPase (and therefore inositol depletion), then one would expect ebselen's effects to be circumvented by administration of exogenous inositol. This reversal is diagnostic of the 'inositol depletion hypothesis' if the addition of inositol re-establishes normal phosphatidylinositol 4,5-bisphosphate signalling[7,8,38]. We injected inositol intracerebroventricularly (Fig. 4a), and this reversed the effects of ebselen in models of both rearing (Fig. 4b) and amphetamine-induced mobility (Fig. 4c). Furthermore, mice injected intraperitoneally with ebselen, showed a decrease in brain levels of inositol 1 h after administration (Fig. 4d,e) providing direct evidence for inositol depletion. Combined, these results are consistent with the known ability of inositol to reverse the behavioural effects of lithium[8,9,38] and support the inositol depletion hypothesis[7].

Discussion

Despite 60 years of use since its discovery as a mood stabilizer[4], lithium remains the gold standard for the treatment of bipolar disorder[1]. Although combination therapy with an antidepressant is used to treat bipolar disorder, there is a risk of precipitating mania[39]. Uniquely, lithium is the only drug reported to reduce suicidal thoughts and behaviour[2]. Lithium is less than ideal, however, due to its undesirable side effects and toxicity. Therefore, there is a crucial need for drugs that are safe and efficacious for the treatment of bipolar disorder. Currently, drugs fail clinical trials for primarily safety or efficacy[40]. Ebselen, is known to be clinically safe[34,41] and hence its efficacy should be tested.

Ebselen exhibits lithium-like actions at many levels including enzymatic, inositol recycling and animal behaviour making it the best lithium mimetic reported to date[5]. Additionally, ebelen is bioavailable, blood-brain barrier permeant and safer than lithium based on cellular toxicity[42] and Phase 1-3 clinical trials[34,41]. Ebselen exhibits a polypharmacology profile (http://mli.nih.gov/mli/mlp-probes/) that would be predicted to be beneficial in its role as a lithium mimetic because it directly inhibits the putative therapeutic targets of lithium including IMPase and protein kinase C[43] as well as being an antioxidant and inhibitor of cyclooxygenases that promotes neuronal survival[44]. Polypharmacology is much more common than previously appreciated[45]. Moreover, polypharmacology is now a desirable property[46,47], for example, antipsychotics hitting multiple targets were more efficacious than drugs that were selective[48].

Inhibition of IMPase by ebselen is covalent and irreversible. Traditionally, covalent drugs have been disfavoured due to risks relating to immunogenicity[49]. However, covalent binding alone is not sufficient to cause problems[50] and many marketed drugs act covalently[49], demonstrating that such risks are compound specific. Importantly, ebselen has a good safety profile in all animal and human experiments reported to date[34,41]. Moreover, the irreversible action of ebelen on IMPase offers several advantages, as is that case for all irreversible drugs[49,51,52]. One is that an irreversible inhibition cannot be overcome by accumulation of substrate. Additionally, irreversible inhibition can interact with pharmacokinetic effects to prolong ebselen's duration of action and increase its selectivity for IMPase. After dosing, the decreasing concentration of ebselen will decrease its inhibition of all its reversible secondary targets. In contrast, IMPase will remain inhibited until new enzyme is synthesized. Such a scenario is known to be the case for many marketed drugs that are covalent and irreversible inhibitors including the well known drugs aspirin, clopidogrel and omeprazole[49].

There is an increasingly urgent need for new drugs for the treatment of mental illness, especially given that many large pharmaceutical companies have pulled out of these areas due to high costs and failure rates[39,53] prompting speculation as to where new drugs will come from for treating disorders of the central nervous system[54,55]. Although there is no single solution, repurposing of drugs from their original use to a new use is being strongly promoted by government initiatives such as that announced by the NIH[16,56,57]. Given that ebselen has been in clinical trials[34,41], ebselen offers all the promise of drug repurposing[58].

Methods

Recombinant Inositol Monophosphatase

Murine *MmImpa*1 and human *HsImpa*1 were amplified from cDNA clones (IMAGE clones 6413389 and 3682657, respectively; Source Bioscience, Cambridge, UK). Cloning and protein expression were carried out as reported[21,59]. Semi-purified recombinant protein was obtained by heating lysed-cell supernatant (68°C, 1 h) and centrifugation (30,000 g, 30 min, 4°C).

Cysteine 218 to Alanine Mutation in IMPase

Site directed mutagenesis of cysteine 218 was carried out using the Stratagene QuickChange kit. Protein was expressed as before, but without sorbitol and betaine.

IMPase Activity

Phosphate hydrolyzed from Ins1P was detected using the malachite green assay. For the in vitro assays, recombinant *Hs*IMPase (10 ng/well) or *Mm*IMPase (30 ng/well) was incubated (1 h, 37°C) with Ins1P (1mM) in 20 μL Tris buffer (50 mM Tris-HCl, 1 mM EGTA, 3 mM $MgCl_2$, 150 mM KCl, 0.5 mg/mL BSA and 0.01% *v/v* Triton X pH 7.4). Absorbance was measured at 595 nm for samples and phosphate standards. For the *ex vivo* assays, brain homogenate (0.5 mg/mL) was incubated (37°C, 1 h) with Ins1P (0.1-2.4 mM) in the presence or absence of LiCl (30 mM) to determine IMPase-specific activity.

Chemical Library and Screening

The NIH Clinical Collection of 450 compounds was provided by the National Institute of Health[16] and purchased from BioFocus DPI. Compounds (100 μM) were screened at three concentrations of Ins1P. Initial hits were confirmed with concentration–inhibition curves spanning six orders of magnitude. Subsequent experiments used ebselen from Fisher Scientific. For compound screening, compound at 100 μM (in 0.2% *v/v* DMSO) was incubated with IMPase (10 min, room temperature) in buffer, before addition of Ins1P (1 mM) to a final volume of 20 μL and further incubated (37°C, 1 h). Phosphatase

concentration was determined by the malachite green assay. LiCl and L-690,330 (Tocris) were used as positive controls.

Reduced Ebselen

Ebselen (250 μM) was treated with 0.25 M dithiothreitol (DTT) or 5 mM GSH; reduced ebselen (final concentration 50 μM) was incubated (1 h, room temperature) with *Hs*IMPase before addition of Ins1P (0.1-3 mM) and further incubation (1 h, 37°C). Enzyme activity was determined by the malachite green assay.

Testing for Reversibility of Drug Inhibition

*Hs*IMPase (1 μg/well) was incubated with 20 μM drug for varying times before dilution to 10 ng/well, addition of Ins1P and further incubation (1 h, 37°C). Enzyme activity was determined by the malachite green assay.

Selenyl-Sulfur Reversibility

Conditions were as described above, except that 2 μL of reductant (50 mM DTT or 1 mM GSH) was added to each well, after incubation of IMPase with ebselen.

Mass Spectrometry

IMPase (100 μM) was desalted using a Bio-Spin 6 Column (Bio-Rad) in 15 mM ammonium acetate (pH 7.5) and incubated (room temperature, 15 min) with 10 mM $MgCl_2$ prior to non-denaturing electrospray ionization mass spectrometry analysis (Q-TOF micro, Micromass). Data were processed with MASSLYNX 4.0 (Waters). To investigate IMPase ligand binding, mass spectrometry was used as described[60], but with an additional mild denaturing step. *Hs*IMPase (100 μM) was incubated with 100 μM drug (10 min) then diluted (1:10) in 15 mM ammonium acetate buffer (pH 7.4) with 0.1% *v/v* formic acid. This solution was then subjected to mass spectroscopy.

Animals

All studies used 20-25 g 10-12 week old male C57Bl6 mice (Harlan Olac, UK). Mice were housed with 12 h light-dark cycles with free access to standard lab chow and water. Experiments were carried out in accordance with UK Home Office Animals (Scientific Procedures) Act (1986) and associated code of practice guidelines. Animals were dosed intraperitoneally (i.p.) at 10 μL/g, unless otherwise specified. Lithium was dosed i.p. at 67 μL/g.

Ex Vivo Mouse Brain Homogenate

Mice were injected with ebselen (10 mg/kg) or vehicle (4% *w/v* hydroxypropyl ß-cyclodextrin) and left for varying amounts of time before euthanization by cervical dislocation, or by CO_2 followed by cervical dislocation. Brains were removed and frozen on dry ice immediately. One hemisphere was homogenized using a Precellys 24 bead mill homogenizer (Stretton) and diluted in Tris buffer (50 mM Tris HCl, 3 mM $MgCl_2$, 150 mM KCl, 1 mM EGTA, 0.01% *v/v* Triton X pH 7.4) to a final concentration of 0.5 mg/mL.

Ex vivo Inositol Measurement by Nuclear Magnetic Resonance

Mice were euthanized by cervical dislocation 1 h after administration of ebselen (10 mg/kg) or vehicle (4% *w/v* hydroxypropyl ß-cyclodextrin), then brains were extracted and frozen immediately on dry ice. Brains were weighed then homogenized using a Precellys 24 bead mill homogenizer (Stretton). Acetonitrile was added to homogenate (1:1 *v/v*) to precipitate protein, the sample was centrifuged (13,000×g, 10 min), and the supernatant was prepared

for NMR by lyophilization and reconstitution in D_2O with 0.008% *w/v* 3-(trimethylsilyl)propionic 2233d acid sodium salt (600 mg/mL).

Amphetamine-induced Hyperactivity

Mice were treated with ebselen or vehicle and immediately placed in Linton AM1053 X, Y, Z IR Activity Monitor (San Diego Instruments) for 1 h to habituate. Mice were then injected with D-amphetamine/saline and returned to the cage, and activity was monitored for an additional 1 h.

Rearing behavior

Mice were injected with ebselen (10 mg/kg) or vehicle (4% *w/v* hydroxypropyl ß-cyclodextrin) and left for varying amounts of time before being placed in the Linton AM1053 X, Y, Z IR Activity Monitor (San Diego Instruments) for 30 mins while their activity was monitored. Rearing was measured by counting the number of beam breaks in upper grid.

Intracerebroventricular Injection of Inositol

Inositol reversal experiments were performed as described[38]. With mice under isoflurane-induced general anaesthesia, a guide cannula was stereotaxically implanted to 1 mm above the injection site in the lateral ventricle, and held in place with dental cement (Aqualox). Mice were left to recover for 7 days before behavioural studies were carried out. Inositol (5 or 1 μL of a supersaturated solution ≥ 278 mM) or control (0.9% *w/v* NaCl) was injected intracerebroventicularly, then 24 h later amphetamine-induced hyperactivity was assessed as described above. Group means were compared with pre-planned t-tests, one-tailed or paired as appropriate.

DOI-induced Head Twitches

Mice were placed in an arena and left to acclimatize to the novel environment. After 1 h, they were injected with vehicle or ebselen (5 or 10 mg/kg) followed 1 h later by the non-selective $5HT_{2A}$ agonist 1- (2,5-dimethoxy-4 iodophenyl)-2-aminopropane (DOI, 2 mg/kg). Head twitches were recorded 5 min after agonist injection for 15 min. Mice were constantly monitored by a video camera, and behavioural recordings were analysed offline independently by two observers who were blind to the treatment.

In Situ Hybridization

For quantification of *Arc* mRNA, brains were removed 1 h after the last injection of drug or vehicle and snap frozen in isopentane cooled by dry ice. Brain tissue sections (12 μm) were cut in a cryostat (-21°C), thaw-mounted onto gelatine-subbed slides and stored (-80°C), then pretreated using standard methods. For *in situ* hybridisation, oligonucleotides complimentary to *Arc* mRNA were 3′-tail labelled with [^{35}S]dATP and applied to each section in hybridization buffer (1×10^{-6} cpm/section). After overnight incubation at 37°C, sections were washed in buffer (3M NaCl and 300 mM citrate, pH 7) first at 55°C (3×20 min) then at room temperature (2×60 min). Sections were then allowed to dry overnight and exposed to Kodak Biomax MR film (Sigma–Aldrich) for 7 days at room temperature. Films were developed and analysed with a computerized image analysis system using densitometric software (MCID, Linton, UK).

Statistical Analyses

Means were compared with pre-planned t-tests (one-tailed or paired as appropriate) using GraphPad Prism software. All data are presented as mean ± standard error of the mean.

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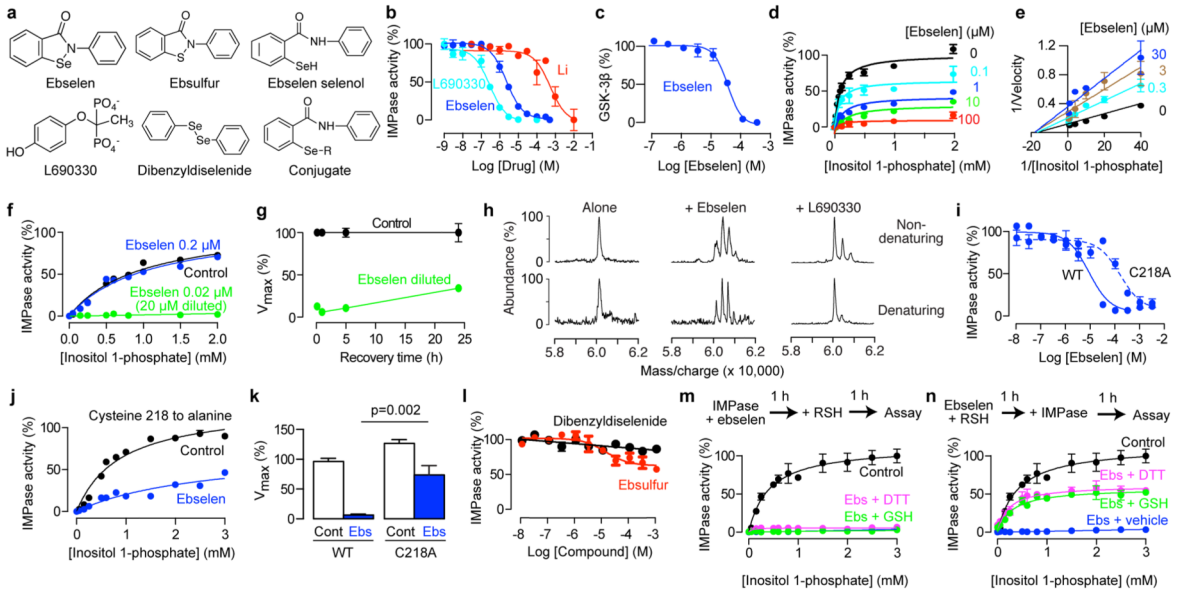

Fig. 1. Ebselen inhibits inositol monophosphatase *in vitro*

(**a**) Chemical structures. For the ebselen conjugate, R is glutathione or another ebselen molecule. (**b**) Concentration–inhibition relationships for ebselen and known inhibitors of IMPase. Assay used expressed human IMPase. (**c**) Concentration–inhibition relationships for ebselen on glycogen synthase kinase 3ß. (**d,e**) Effect of ebselen on the enzyme kinetics of IMPase shown as a Michaelis-Menten plot (**d**) or a Lineweaver-Burk plot (**e**). (**f**) Effect of dilution on inhibition of IMPase by ebselen (20 μM before and 0.2 μM after dilution). (**g**) Effect of recovery time after dilution on inhibition of IMPase by ebselen. (**h**) Mass spectroscopy under mild denaturing and non-denaturing conditions of IMPase incubated with ebselen or L-690,330 (100 μM each). (**i-k**) Effect of mutation cysteine-218 to alanine on inhibition of IMPase by ebselen assessed by concentration-inhibition curves (**i**), Michaelis-Menton kinetics (ebselen 50 μM) (**j**) and V_{max} (**k**). Analyzed by a pre-planned t-test, n=6. (**l**) Concentration-response relationships for the sulfur analogue of ebselen and dibenzyldiselenide on IMPase. (**m,n**) Effect of disulfide reducing agents on inhibition of IMPase by ebselen (50 μM) with either post-incubation with glutathione (GSH, 1 mM) or dithiothreitol (50 mM) (**m**) or pre-incubation with 5 mM and 250 mM, respectively (**n**). All error bars represent standard error of the means.

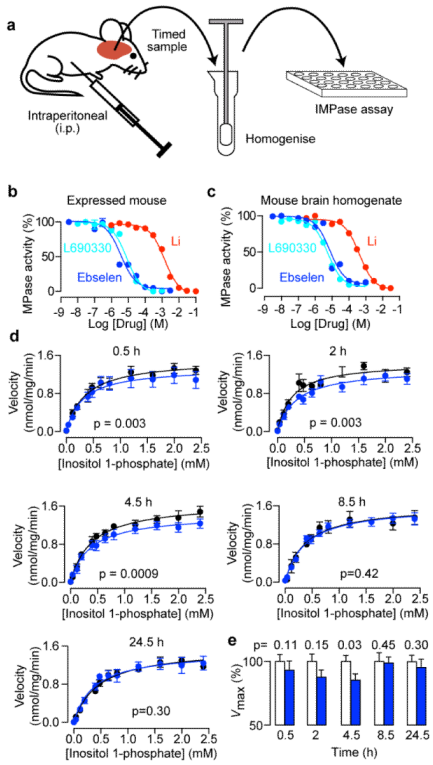

Fig. 2. Ebselen permeates the blood-brain barrier and inhibits endogenous inositol monophosphatase in mouse brain

(**a**) Schematic illustrating the experimental protocol for assessing IMPase in *ex vivo* and brain homogenate experiments. Ebselen was injected at 10 mg/kg. (**b,c**) Concentration-inhibition relationships for novel and known inhibitors of mouse IMPase expressed in bacteria (**b**) or present in homogenates from mouse brain (**c**). (**d**) Michaelis-Menten plots showing the effect of injected ebselen on IMPase in *ex vivo* brain homogenate. Statistical significance was determined by a global fit of the Michaelis-Menten equation to the entire data set. (**e**) Effect of ebselen on the V_{max} of IMPase over time after injection, analysed by pre-planned paired t-test between the treatment and control, n=5-6. All error bars represent standard error of the means.

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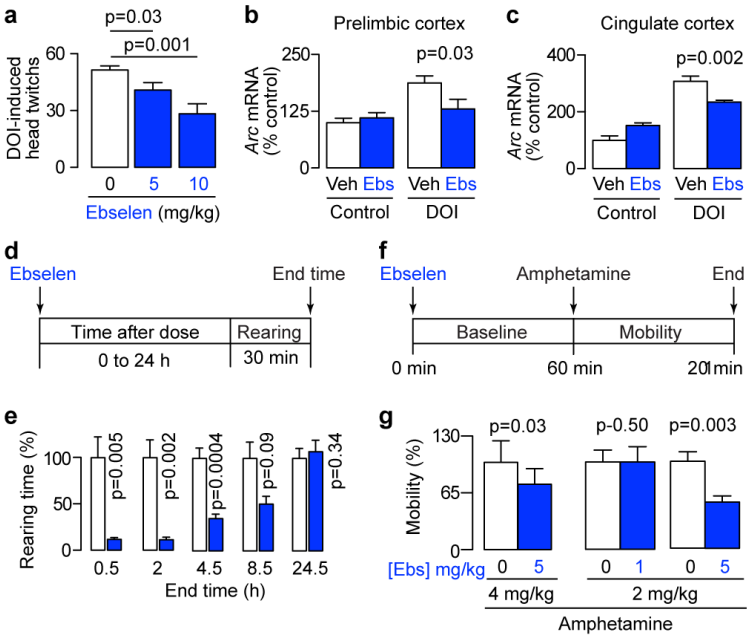

Fig. 3. Ebselen induces lithium-like behaviour

(**a**) Ebselen attenuates the head-twitch response induced by the 5-HT_{2A} agonist DOI (2 mg/kg), analyzed by pre-planned, one-tailed t-tests, n=6. (**b, c**) Ebselen (10 mg/kg) attenuates the increase in the immediate-early gene *Arc* mRNA induced by DOI (2 mg/kg) in two cortical regions. Analyzed by pre-planned, one-tailed t-tests, n=5-6. (**d, f**) Experimental protocols for assessing the effect of ebselen on behaviour in the open field test during exploratory activity (rearing, **d**) and amphetamine-induced hyperactivity (mobility, **f**). (**e**) Effect of ebselen (10 mg/kg) on rearing over time, analyzed by pre-planned paired t-tests, n=5-6. (**g**) Effect of ebselen on mobility during amphetamine-induced hyperactivity, analyzed by pre-planned paired t-test, n=6-8. All error bars represent standard error of the means.

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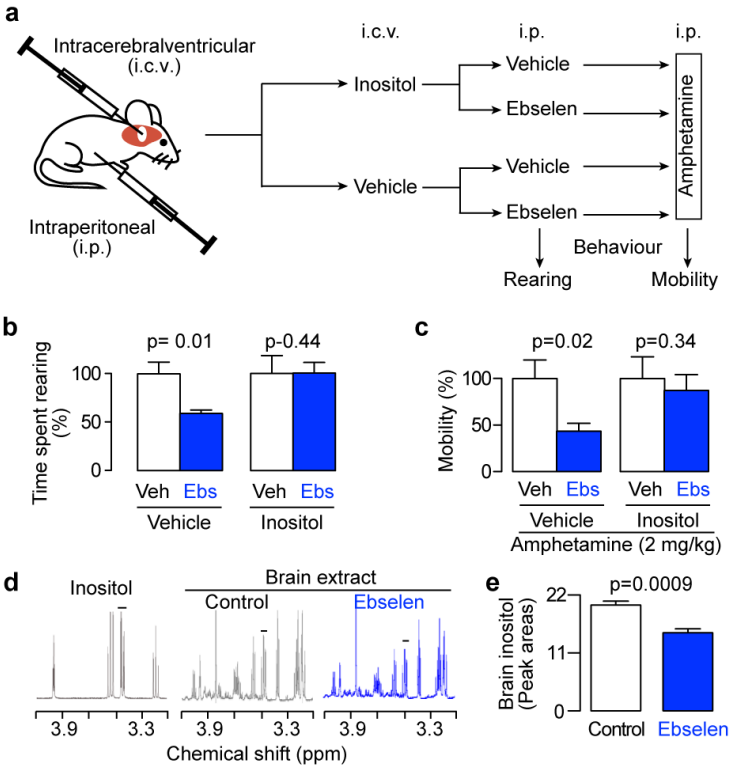

Fig. 4. The pharmacological effects of ebselen are mediated by inositol depletion

(**a**) Schematic outlining the experimental protocol used to investigate inositol reversal of behaviours induced by ebselen. Drugs were injected as follows: 1 μL of 0.5 M inositol, 5 mg/kg ebselen and 2 mg/kg amphetamine. (**b**) Effect of inositol on the ability of ebselen to attenuate rearing, analysed by pre-planned paired t-tests between ebselen and control, n=4-6. (**c**) Effect of inositol on the ability of ebselen to attenuate amphetamine-induced hyperactivity (mobility) analyzed by pre-planned paired t-tests between ebselen and control, n=5-6. (**d**) Proton NMR spectra of authentic inositol and brain extracts from mice injected intraperitoneally with either ebselen (10 mg/kg) or hydroxypropyl ß-cyclodextrin(4% *w/v*, Control). (**e**), Effect of ebselen (10 mg/kg) on inositol levels in mouse brain. Inositol was quantified by integration of the C1 and C3 peaks (indicated by the bar in **d**), and analyzed by a pre-planned one-way t-test between ebselen and control, n=4. All error bars represent standard error of the means.